REMARKS

The Remarks and Amendments herein are responsive to the Final Office Action dated September 2, 2009. Applicant has filed a Request for Continued Examination herewith.

Objection to the Specification Under 35 U.S.C. §132(a)

Pursuant to 35 U.S.C. §132(a), the Examiner objected to a portion of the preliminary amendment filed on April 28, 2009 for allegedly introducing new matter into the specification. Applicant respectfully disagrees with the conclusion of the Examiner regarding the introduction of new matter. In the amendment filed on April 28, 2009, Applicant amended paragraphs [0055] and [0151] of the specification to recite that "△ shows p21 mRNA for the control stimulation ... [and that] ◇ shows FasL mRNA for the control stimulation." (Applicant's Amendment of April 28, 2009, pages 2 and 7). As discussed at the interview, the specification as filed and drawings provide clear support for these statements.

In paragraph [0151] of the specification as filed, Applicant discloses that the responsiveness of leukocytes to PMA and CaI stimulation were assessed by quantitating p21 and FasL mRNA, and discusses the data curves shown in FIG. 22A. Paragraph [0151] (lines 9-11 on page 37) describes the control stimulation curves in Fig 22A by stating that "[t]he levels of p21 were also increased slightly by incubation at 37°C ***without any stimulation***, whereas FasL remained unchanged (FIG. 22A)". One of ordinary skill would recognize this passage not only to be explicitly describing the shape of the two curves with open symbols at the bottom of FIG. 22A, but to be describing control samples, based on the explicit lack of stimulation.

Moreover, each other Example in the specification presented data for control samples with open symbols. For example, paragraph [0150] indicates that the (o) symbol in FIGS 21-B-E are "standard mRNA," while closed symbols are used to represent the target mRNAs, CD4 (•), p21 (▲) , FasL (♦)and Leukotriene C4 synthase (■). Thus, one of ordinary skill would recognize that (i) due to the corresponding shapes of the curves in FIG. 22A using open symbols with the description of the curves of control samples recited in paragraph [0151], and (ii) due to the use of open symbols, (△) for p21 and (◇) for FasL and that the curves would be representative of non-stimulatory (i.e. control) conditions. As such, Applicant submits that the amendments filed on April 28, 2009 did not introduce new matter to the specification, but rather

further clarified the information already disclosed. Applicant therefore respectfully requests withdrawal of the objection to the specification.

Claim Objections

The Examiner objected to Claim 73 for certain informalities in the claim language. Appropriate amendments have been made and Applicant respectfully requests withdrawal of the objection.

Claim Rejections – 35 U.S.C. §112 – first paragraph, new matter

The Examiner rejected Claims 215, 217-219, 231-233 and 240 under 35 U.S.C. §112, first paragraph for failing to meet the written description requirement as the claims allegedly contain new matter not described in the specification as filed.

Specifically, the Examiner rejected Claim 217 because the specification allegedly does not describe "a plurality of different antisense primers for different target mRNAs are present in the lysis buffer." (Office Action, page 3) Applicant respectfully disagrees with the Examiner's conclusion that this portion of Claim 217 constitutes new matter, as the specification as filed provides several passages indicating that the primers are present in the lysis buffer. For example, the specification as filed, at paragraph [0111], indicates that "[s]ince ***primers were added to the lysis buffer***, primers were not included in the cDNA synthesis reaction." Paragraph [0136] also discloses that the "***lysis buffer preferably contains*** a mixture of primers…" Further, paragraph [0094] similarly indicates that "[i]n a preferred embodiment, ***amplification primers are included in the lysis buffer***." Thus, Applicant respectfully submits that the specification describes a plurality of different antisense primers for different target mRNAs that are present in the lysis buffer. As such, Applicant respectfully requests withdrawal of the new matter rejection of Claim 217.

The Examiner also rejected Claim 233 and alleged that the specification does not provide disclosure to support the presented Claim language, which recites that the "target mRNA is mRNA responsible for apoptosis development, and wherein the quantification of mRNA is used to test the side effects of anti-cancer drugs that induce mRNA responsible for apoptosis development." Applicant has amended Claim 233 to indicate that the target mRNA is mRNA responsible for apoptosis development, which, as recognized by the Examiner, is fully supported

in Table 1 and original Claim 29 of the specification. (Office Action, pages 3-4) Therefore, Applicant respectfully requests withdrawal of the new matter rejection of Claim 233.

The Examiner rejected step (h) of Claim 215 as constituting new matter by indicating that that the specification does not describe "quantifying the target mRNA by quantifying the cDNA in said cDNA solution." Applicant has amended step (h) of Claim 215 to recite "quantifying the target mRNA by ***amplifying the cDNA in said cDNA solution*** and quantifying the ***amplified cDNA*** from said cDNA solution." Support for the amended Claim language can be found generally in the specification as filed at paragraphs [0082] – [0085], which disclose various amplification and quantitation techniques, as well as throughout the Examples. Therefore, Applicant respectfully submits that step (h) of Claim 215 as amended does not recite new matter, and respectfully requests withdrawal of the new matter rejection of Claim 215.

The Examiner also rejected Claim 218 because the specification allegedly does not describe "each of said different mRNAs is amplified from the cDNA formed by extension of the antisense primers in step (g) [of Claim 215,]" as well as Claim 240 because the specification allegedly does not describe "each of said different mRNAs is amplified from the cDNA formed by extension of the immobilized oligo(dT) in step (g) [of Claim 215]." Applicant respectfully disagrees with the Examiner's conclusion that Claims 218 and 240 constitute new matter, as the specification as filed indicates that mRNAs are amplified from cDNA formed by extension of the antisense primers or the immobilized oligo(dT). For example, in paragraph [0080], Applicant discloses that:

> "[t]he oligo(dT) and the specific primer (NNNN) simultaneously prime cDNA synthesis at different location on the poly-A RNA (FIG. 15). The specific primer (NNNN) and oligo(dT) cause the formation of cDNA during amplification, as shown in FIG 15."

Applicant respectfully submits that in light of the amendment discussed above with respect to Claim 215, the disclosure of paragraph [0080], which specifically discusses the amplification of mRNAs from cDNA formed by extension ***both*** of the antisense primers or the immobilized oligo(dT), particularly when read in conjunction with FIGS. 15 and 16, that the original disclosure fully supports both Claim 218 and 240. As such, Applicant respectfully requests withdrawal of the new matter rejections for Claims 218 and 240.

Claim Rejections – 35 U.S.C. §112 – second paragraph, indefiniteness

The Examiner rejected Claims 73, 75, 77-88, 91, 92, 215, 217-219, 231-233, and 240 under 35 U.S.C. §112, as being indefinite for allegedly failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The Examiner rejected Claim 73 as being indefinite because (i) it is unclear that the definite quantity of the target mRNA is the quantity of total mRNA from leukocytes or that quantity of specific mRNA encoding for a specific gene, and (ii) step (d) of Claim 73 does not require that the spiked control RNA is mRNA, and as such the percent recovery of the spiked control RNA is not comparable to that of the target mRNA.

Applicant has amended Claim 73 to clarify that the definite quantity of the target mRNA is the quantity of specific mRNA encoding a specific gene. Amended Claim 73 now recites a method of "determining a definite quantity of a target mRNA ***encoding a specific sequence*** in a blood sample..." and in step (h) "determining the definite quantity of ***said*** target mRNA..." Support for this amendment may be found at paragraph [0083] of the specification, where it is indicated that the detection of target sequences is sequence-specific.

Applicant has also amended step (d) of Claim 73 to indicate more clearly that the spiked control RNA is spiked control ***poly-A*** RNA, thus allowing the percent recoveries to be accurately compared. In light of the above amendments, Applicant respectfully submits that the rejections of Claim 73 have been addressed and requests withdrawal of the rejections. Applicant also requests withdrawal of the rejections of Claim 75, 77-88, 91, 92, and 231-233, each of which depend directly or indirectly from Independent Claim 73.

The Examiner also rejected Claim 215 as indefinite in view of steps (g) and (h), and Claim 218 as indefinite as it depended from step (g) of Claim 215. The Examiner indicated that since step (g) does not require the presence of a DNA polymerase with 3' to 5' exonuclease activity, it was unclear to the Examiner how the cDNA formed by extension of the antisense primers can go into solution as a result of displacement by the strand extended from the oligo(dT). The Examiner also indicated that, if a DNA polymerase with 3' to 5' exonuclease activity were present, the displaced cDNA would be fragmented due to the exonuclease, thus making it unclear how target mRNA could be quantified by quantifying the cDNA in the solution.

Applicants respectfully disagree with the Examiner's conclusion regarding Claims 215 and 218, as the specification as filed and the Examples therein fully describe and render the Claims definite. As discussed in the interview and in paragraph [0080], Example 6, and the associated data in FIGS 15 and 16 disclose the protocol and results that describe the steps of Claim 215. Paragraph 80 discloses that specific primers (NNNN) added in the lysis buffer and the immobilized oligo(dT) simultaneously prime cDNA synthesis at different locations on the poly-A RNA. This is depicted in FIG 15 as a poly-A RNA having bound to it an "NNNN" primer sequence and also being bound to "TTTTTTT," which is immobilized. Reverse transcriptase enzymes recognize double stranded regions of nucleic acid and therefore simultaneously synthesizes cDNA from the oligo(dT)-poly A site, as well as the site of specific primer (NNNN) hybridization. This is depicted in the middle portion of FIG 15. As cDNA synthesis advances, the leading edge of the cDNA strand resulting from elongation from the oligo(dT)-poly A site will reach the site where the specific primer (NNNN) is hybridized to the mRNA strand. As explicitly stated in paragraph [0080], "one possible explanation for such results is that oligo(dT)-derived cDNA may displace primer-derived cDNA during amplification," which is depicted in the lower portion of FIG 15. Further, FIG 16 shows experimental data that confirms that PCR amplification of primer-derived cDNA yields similar amounts of specific product, whether a heat-denaturing step is or is not used. This demonstrates that the oligo(dT)-derived cDNA is in fact displacing the specific primer (NNNN)-derived cDNA strand during cDNA synthesis.

Finally, as discussed in paragraphs [0132] – [0133] (Example 6) and the associated FIG 17, cDNA synthesis was performed in the presence or absence of gene specific primers (for CD4). Amplification of the CD4 gene was then performed from the solution in the sample wells and directly from the Gene plate. As shown in FIG 17, CD4 was amplified from both samples, further demonstrating the displacement of the upstream specific primer (NNNN) by the oligo(dT)-derived cDNA.

In light of the demonstration in the specification and corresponding Examples as described above, Applicant respectfully submits that step (g) of Claim 215 is not indefinite. The specification and Examples present and demonstrate the validity of a possible mechanism by which a cDNA strand may be displaced into solution during synthesis by a reverse transcriptase enzyme, and without the need for exonuclease activity. Applicant respectfully submits that

because there is no need for, nor inclusion of, a DNA polymerase in the steps recited in Claim 215, the consideration of how target mRNA fragmented by a 3' to 5' exonuclease activity could be quantified by quantifying the cDNA in the solution is moot. As such Applicant respectfully requests that the indefiniteness rejections of Claims 215 and 218 be withdrawn. Applicant also requests that the rejections of Claims 217 and 219, and 240, each of which depend either directly or indirectly from Claim 215, be withdrawn.

In light of the above amendments and remarks, Applicant respectfully submits that each objection and rejection presented by the Examiner has been addressed and overcome. As such, Applicant respectfully submits that all Claims are in condition for allowance.

No Disclaimers or Disavowals

Although the present communication may include alterations to the application or claims, or characterizations of claim scope or referenced art, Applicant is not conceding in this application that previously pending claims are not patentable over the cited references. Rather, any alterations or characterizations are being made to facilitate expeditious prosecution of this application. Applicant reserves the right to pursue at a later date any previously pending or other broader or narrower claims that capture any subject matter supported by the present disclosure, including subject matter found to be specifically disclaimed herein or by any prior prosecution. Accordingly, reviewers of this or any parent, child or related prosecution history shall not reasonably infer that Applicant has made any disclaimers or disavowals of any subject matter supported by the present application.

Co-Pending Applications of Assignee

Applicant wishes to draw the Examiner's attention to the following applications of the present application's assignee, which are currently pending unless indicated otherwise.

Docket No.	Serial No.	Title	Filed
HITACHI.031A (issued)	10/048800 (6,844,158)	DIRECT RT-PCR ON OLIGONUCLEOTIDE IMMOBILIZED PCR MICROPLATES	Jan. 31, 2002 (Jan. 18, 2005)
HITACHI.031DV1 (abandoned)	10/404988	KIT FOR DIRECT RT-PCR ON OLIGONUCLEOTIDE IMMOBILIZED PCR MICROPLATES	Mar. 31, 2003
HITACHI.031DV2 (issued)	10/404033 (7,258,976)	METHODS OF PREPARING CELL LYSATE	Mar. 31, 2003 (Aug. 21, 2007)

HITACHI.055CP1 (abandoned)	10/698967	DEVICE AND METHOD FOR HIGH-THROUGHPUT QUANTIFICATION OF MRNA FROM WHOLE BLOOD	Oct. 30, 2003
HITACHI.055CP2C1	11/525515	DEVICE AND METHOD FOR HIGH-THROUGHPUT QUANTIFICATION OF MRNA FROM WHOLE BLOOD	Sept. 22, 2006
HITACHI.55CP2C2	11/376,018	DEVICE AND METHOD FOR HIGH-THROUGHPUT QUANTIFICATION OF MRNA FROM WHOLE BLOOD	March 15, 2006
HITACHI.55CP2D1	11/803593	DEVICE AND METHOD FOR HIGH-THROUGHPUT QUANTIFICATION OF MRNA FROM WHOLE BLOOD	May 15, 2007
HITACHI.55CP2D2	11/803594	DEVICE AND METHOD FOR HIGH-THROUGHPUT QUANTIFICATION OF MRNA FROM WHOLE BLOOD	May 15, 2007
HITACHI.55CP2D3	11/803663	DEVICE AND METHOD FOR HIGH-THROUGHPUT QUANTIFICATION OF MRNA FROM WHOLE BLOOD	May 15, 2007

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

Respectfully submitted,

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